09/857307

PCT

GENERAL POWER OF ATTORNEY

(for several international applications filed under the Patent Cooperation Treaty)

(PCT Rule 90.5)

The undersigned person(s) :
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

PURDUE RESEARCH FOUNDATION
1021 Hovde Hall, Room 307
West Lafayette, Indiana 47907-1021
US

hereby appoint(s) the following person as: [XX] agent [ ] common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

LAMMERT, Steven R.; COFFEY, William R.; HYLAND, Jerry E.; CONARD, Richard D.; REZEK, Richard A.; NIEDNAGEL, Timothy E.; BREEN, John P.; WOODBURN, Jill L.; HARRISON, Nancy, J.; CARTER, R. Trevor; KULKARNI, Dilip A.; BARRY, Michael M.; QUICK, David B.; POWLICK, Jill T.; PALAN, Perry; NEWMAN, Mark M.; GILLENWATER, Bobby B.; HUNT, Paul B.; GZYBOWSKI, Michael S.; GALLAGHER, Gerald T.; NULL, Robert D.; All Appointed Agents of the Address:
BARNES & THORNBURG
11 South Meridian Street
Indianapolis, Indiana 46204
US

to represent the undersigned before

[XX] all the competent International Authorities
[ ] the International Searching Authority only
[ ] the International Preliminary Examining Authority only

in connection with any and all international applications filed by the undersigned with the following Office

US as receiving Office

and to make or receive payments on behalf of the undersigned.

Signature(s) *(where there are several persons, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading this power):*

Bruce L. Pershing
Signature

Date: (08.01.99) 08 January 1999
Day/ Month/ Year

Printed Name: Bruce L. Pershing
Title: Investment Officer and Secretary

Form PCT/Model of general power of attorney (for several international applications) (July 1992)

09/857307

PCT

GENERAL POWER OF ATTORNEY

(for several international applications filed under the Patent Cooperation Treaty)

(PCT Rule 90.5)

The undersigned person(s):
(Family name followed by given name; for a full legal entity, full official designation. The address must include postal code and name of country.)

BADYLAK, Stephen F.
1150 Kingswood Rd. S.
West Lafayette, IN 47906
US

hereby appoint(s) the following person as: ☒ agent ☐ common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

LAMMERT, Steven R.; REZEK, Richard A.; COFFEY, William R.; CONARD, Richard D.; NIEDNAGEL, Timothy E.; HARRISON, Nancy J.; CARTER, R. Trevor; WAITE, Kenneth J.; KULKARNI, Dilip A.; QUICK, David B.; POWLICK, Jill T.; HEDGES, Norman J.; PALAN, Perry; NEWMAN, Mark M.; GILLENWATER, Bobby B.; HUNT, Paul B.; GZYBOWSKI, Michael S.; GALLAGHER, Gerard T.; NULL, Robert D.; MARTIN, Alice O.; and COOPER, Gregory S. all of the law firm: BARNES & THORNBURG
11 South Meridian Street
Indianapolis, IN 46204
US

to represent the undersigned before

☒ all the competent International Authorities

☐ the International Searching Authority only

☐ the International Preliminary Examining Authority only

in connection with any and all international applications filed by the undersigned with the following Office

US as receiving Office

and to make or receive payments on behalf of the undersigned.

Signature(s) *(where there are several persons, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading this power):*

Stephen F. Badylak

Stephen F. BADYLAK, Co-Applicant

Date: 10/07/99

65477

09/857307

PCT

GENERAL POWER OF ATTORNEY

(for several international applications filed under the Patent Cooperation Treaty)

(PCT Rule 90.5)

The undersigned person(s) :
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

SPIEVACK, Alan R.
6 Old Dee Road
Cambridge, MA 02138
US

hereby appoint(s) the following person as: [XX] agent [ ] common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)
LAMMERT, Steven R.; COFFEY, William R.; CONARD, Richard D.; REZEK, Richard A.; NIEDNAGEL, Timothy E.; HARRISON, Nancy, J.; CARTER, R. Trevor; KULKARNI, Dilip A.; QUICK, David B.; POWLICK, Jill T.; HEDGES, Norman J.; STEIN, Arland T.; RICHARDS, William B.; WAITE, Kenneth J.; REYNOLDS, Thomas S. III; PALAN, Perry; NEWMAN, Mark M.; GILLENWATER, Bobby B.; HUNT, Paul B.; GZYBOWSKI, Michael S.; GALLAGHER, Gerald T.; NULL, Robert D.; MARTIN, Alice O.; COOPER, Gregory S.; All Appointed Agents of the Address:
BARNES & THORNBURG
11 South Meridian Street
Indianapolis, IN 46204
US

to represent the undersigned before

[XX] all the competent International Authorities
[ ] the International Searching Authority only
[ ] the International Preliminary Examining Authority only

in connection with any and all international applications filed by the undersigned with the following Office

US as receiving Office

and to make or receive payments on behalf of the undersigned.

Signature(s) *(where there are several persons, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading this power):*

Alan R. Spievack
Alan R. SPIEVACK

Date: 12 17 99
Day/ Month/ Year

Form PCT/Model of general power of attorney (for several international applications) (July 1992)

PATENT COOPERATION TREATY

PCT

09/857307

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
3220-65477		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 28300	01/12/1999	01/12/1998
Applicant		
PURDUE RESEARCH FOUNDATION et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. --

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/28300

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-10 are directed to a method of treatment of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box 1.1

Although claims 1-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box 1.1

Rule 39.1(iv) PCT - Method for treatment of the humanr/animal body by surgery

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/28300

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 10775 A (BADYLAK STEPHEN F ;COBB MARK A (US); ISOM GARY (US); SHARMA ARCHAN) 19 March 1998 (1998-03-19)	9,11
A	example 2 claims	1-3,5-8, 11
Y	ISSHIKI N ET AL: "Surgical treatment of laryngeal web with mucosa graft" ANNALS OF OTOLOGY, RHINOLOGY AND LARYNGOLOGY, vol. 100, 1991, pages 95-100, XP000901865 page 95, column 2, line 10 - line 16 page 99, column 2, last paragraph figure 2	1-11

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 April 2000	26/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Thornton, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 25637 A (BADYLAK STEPHEN F ;PURDUE RESEARCH FOUNDATION (US)) 18 June 1998 (1998-06-18) page 3, line 3 -page 5, line 2 page 6, line 4 - line 9 page 12, line 3 -page 13, line 6 claims	1-11
A	US 5 573 784 A (BADYLAK STEPHEN F ET AL) 12 November 1996 (1996-11-12) column 1, line 16 - line 55 claim 1	1-3,5-11
A	WO 98 40027 A (GERIGENE MEDICAL CORP ;KLEINSEK DON A (US)) 17 September 1998 (1998-09-17) page 28, line 15 -page 29, line 25 claims	1,9-11
A	WO 96 40175 A (ADVANCED TISSUE SCIENCES INC) 19 December 1996 (1996-12-19) page 50, line 19 -page 53, line 16 claims 1-6,10	1,5-11
A	PANKRATOV M ET AL: "Endoscopic diode-laser applications in airway surgery" PROC SPIE INT SOC OPT ENG. PROCEEDINGS OF SPIE - THE INTERNATIONAL SOCIETY FOR OPTICAL ENGINEERING. PROCEEDINGS OF LASER SURGERY: ADVANCED CHARACTERIZATION, THERAPEUTICS, AND SYSTEMS IV, vol. 2128, 1994, pages 33-40, XP000901390 ISSN 0277-786X ISBN 0-8194-1421-2 page 33, last paragraph -page 34, line 10 page 37, line 24 -page 38, line 16 page 38, last paragraph	1,9,10

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/28300

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9810775 A	19-03-1998	AU	4348597 A	02-04-1998
		EP	0925067 A	30-06-1999
WO 9825637 A	18-06-1998	AU	5695898 A	03-07-1998
		EP	0942739 A	22-09-1999
US 5573784 A	12-11-1996	US	5445833 A	29-08-1995
		US	5281422 A	25-01-1994
		AU	668520 B	09-05-1996
		AU	2651192 A	27-04-1993
		CA	2119750 A	01-04-1993
		EP	0605581 A	13-07-1994
		JP	6510927 T	08-12-1994
		MX	9205388 A	01-05-1993
		NZ	244475 A	26-05-1995
		WO	9305798 A	01-04-1993
		US	5372821 A	13-12-1994
WO 9840027 A	17-09-1998	AU	6334498 A	29-09-1998
		AU	6661698 A	09-09-1998
		AU	6662698 A	09-09-1998
		WO	9836704 A	27-08-1998
		WO	9836705 A	27-08-1998
WO 9640175 A	19-12-1996	US	5863531 A	26-01-1999
		AU	706426 B	17-06-1999
		AU	6031596 A	30-12-1996
		CA	2224071 A	19-12-1996
		EP	0831861 A	01-04-1998
		JP	11506611 T	15-06-1999
		NZ	310004 A	28-10-1999
		US	6022743 A	08-02-2000

09/857307

REC'D 16 JAN 2001

WIPO PCT

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

15

Applicant's or agent's file reference 3220-65477	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US99/28300	International filing date *(day/month/year)* 01 DECEMBER 1999	Priority date *(day/month/year)* 01 DECEMBER 1998
International Patent Classification (IPC) or national classification and IPC IPC(7):A61B 19/00 and US Cl.: 128/898		
Applicant PURDUE RESEARCH FOUNDATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08 JUNE 2000	12 DECEMBER 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer DAVID J ISABELLA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-3060

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRE[redacted]NARY EXAMINATION REPORT

Int[redacted]ional application No.
PCT/US99/28300

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-16 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 17-18 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELI[redacted]RY EXAMINATION REPORT

Int[redacted]onal application No.
PCT/US99/28300

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-11	YES
	Claims	NONE	NO
Inventive Step (IS)	Claims	1-11	YES
	Claims	NONE	NO
Industrial Applicability (IA)	Claims	1-11	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-11 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest a surgical method for repair of the vocal cord tissues by replacing the damaged tissue with a graft construct derived from the vertebrate submucosa or basement membrane.

---------- NEW CITATIONS ----------

US 5,549,673 A (BEALE) 27 AUGUST 1996, ENTIRE PUBLICATION

US 5,762,966 A (KNAPP, JR. ET AL) 9 JUNE 1998, ENTIRE PUBLICATION

Express Mail No.: EL230257915US Mailed: May 2001

09/857307

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 3220-65477

Box No. I TITLE OF INVENTION

METHOD FOR VOCAL CORD RECONSTRUCTION

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PURDUE RESEARCH FOUNDATION
1021 Hovde Hall, Room 307
West Lafayette, IN 47907-1021
US

☐ This person is also inventor.

Telephone No. (765) 494-2610

Facsimile No. (765) 496-1277

Teleprinter No.

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BADYLAK, Stephen F.
1150 Kingswood Road South
West Lafayette, IN 47906
US

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LAMMERT, Steven R.
BARNES & THORNBURG
11 South Meridian Street
Indianapolis, IN 46204
US

Telephone No. (317) 236-1313

Facsimile No. (317) 231-7433

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SPIEVACK, Alan R.
6 Old Dee Road
Cambridge, MA 02138
US

This person is:

- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- [X] **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [X] **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] **AE** United Arab Emirates
- [X] **AL** Albania .....
- [X] **AM** Armenia .....
- [X] **AT** Austria .....
- [X] **AU** Australia .....
- [X] **AZ** Azerbaijan
- [X] **BA** Bosnia and Herzegovina .....
- [X] **BB** Barbados
- [X] **BG** Bulgaria .....
- [X] **BR** Brazil .....
- [X] **BY** Belarus .....
- [X] **CA** Canada
- [X] **CH and LI** Switzerland and Liechtenstein
- [X] **CN** China .....
- [X] **CU** Cuba .....
- [X] **CZ** Czech Republic .....
- [X] **DE** Germany .....
- [X] **DK** Denmark .....
- [X] **EE** Estonia .....
- [X] **ES** Spain .....
- [X] **FI** Finland .....
- [X] **GB** United Kingdom
- [X] **GD** Grenada
- [X] **GE** Georgia .....
- [X] **GH** Ghana .....
- [X] **GM** Gambia
- [X] **HR** Croatia .....
- [X] **HU** Hungary .....
- [X] **ID** Indonesia
- [X] **IL** Israel .....
- [X] **IN** India .....
- [X] **IS** Iceland
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- [X] **KE** Kenya .....
- [X] **KG** Kyrgyzstan .....
- [X] **KP** Democratic People's Republic of Korea .....
- [X] **KR** Republic of Korea .....
- [X] **KZ** Kazakhstan .....
- [X] **LC** Saint Lucia
- [X] **LK** Sri Lanka
- [X] **LR** Liberia
- [X] **LS** Lesotho .....
- [X] **LT** Lithuania
- [X] **LU** Luxembourg
- [X] **LV** Latvia
- [X] **MD** Republic of Moldova .....
- [X] **MG** Madagascar .....
- [X] **MK** The former Yugoslav Republic of Macedonia .....
- [X] **MN** Mongolia
- [X] **MW** Malawi .....
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- [X] **SK** Slovakia .....
- [X] **SL** Sierra Leone .....
- [X] **TJ** Tajikistan .....
- [X] **TM** Turkmenistan .....
- [X] **TR** Turkey .....
- [X] **TT** Trinidad and Tobago .....
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- [X] **UG** Uganda .....
- [X] **US** United States of America .....
- [X] **UZ** Uzbekistan .....
- [X] **VN** Viet Nam .....
- [X] **YU** Yugoslavia .....
- [X] **ZA** South Africa .....
- [X] **ZW** Zimbabwe .....

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

- [X] **CR** Costa Rica
- [X] **DM** Dominica .....
- [X] **TZ** Tanzania
- [X] **MA** Morocco .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) (01.12.98) 01 December 1998	60/110,401	US		
item (2) (01.12.98) 01 December 1998	60/110,465	US		
item (3)				

XX The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1) and (2)

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA / EP

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	4
description (excluding sequence listing part)	16
claims	2
abstract	1
drawings	0
sequence listing part of description	0
Total number of sheets	23

This international application is **accompanied by** the item(s) marked below:

1. XX fee calculation sheet
2. XX separate signed power of attorney
3. XX copy of general power of attorney; reference number, if any: (2)
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Transmittal Letter to the US/RO Return Postal Card

Figure of the drawings which should accompany the abstract: None

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Steven R. Lammert

Steven R. Lammert, Agent for Applicants

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

09/857307

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF RECEIPT OF SEARCH COPY

(PCT Rule 25.1)

To:
BARNES & THORNBURG
Attn. LAMMERT, STEVEN R.
11 South Meridian Street
Indianapolis, IN 46204
UNITED STATES OF AMERICA

Date of mailing *(day/month/year)*	21/03/2000
Applicant's or agent's file reference	IMPORTANT NOTIFICATION
3220-65477	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 99/28300	01/12/1999	01/12/1998

Applicant

PURDUE RESEARCH FOUNDATION et al.

1. **Where the International Searching Authority and the Receiving Office are not the same office:**

The applicant is hereby notified that the search copy of the international application was received by this International Searching Authority on the date indicated below.

Where the International Searching Authority and the Receiving Office are the same office:

The applicant is hereby notified that the search copy of the international application was received on the date indicated below.

06/03/2000 *(date of receipt).*

2. ☐ The search copy was accompanied by a nucleotide and/or amino acid sequence listing in computer readable form.

3. **Time limit for establishment of International Search Report**

The applicant is informed that the time limit for establishing the International Search Report is 3 months from the date of receipt indicated above or 9 months from the priority date, whichever time limit expires later

4. A copy of this notification has been sent to the International Bureau and, where the first sentence of paragraph 1 applies, to the Receiving Office.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl Fax: (+31-70) 340-3016	ISA/EP

Form PCT/ISA/202 (July 1998)

****** COPY FOR RECEIVING OFFICE ******

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 13 September 2000 (13.09.00)	
International application No. PCT/US99/28300	**Applicant's or agent's file reference** 3220-65477
International filing date (day/month/year) 01 December 1999 (01.12.99)	**Priority date** (day/month/year) 01 December 1998 (01.12.98)
Applicant BADYLAK, Stephen, F. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 June 2000 (08.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Manu Berrod

Telephone No.: (41-22) 338.83.38

US9928300

Express Mail No. EL230257975US dtd 11/7/00

PCT/US99/28300

PATENT COOPERATION TREATY

09/857307

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

LAMMERT, Steven, R.
Barnes & Thornburg
11 South Meridian Street
Indianapolis, IN 46204
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 19 October 2000 (19.10.00)	
Applicant's or agent's file reference 3220-65477	**IMPORTANT NOTIFICATION**
International application No. PCT/US99/28300	**International filing date** (day/month/year) 01 December 1999 (01.12.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
PURDUE RESEARCH FOUNDATION 1021 Hovde Hall Room 307 West Lafayette, IN 47907-1021 United States of America	US	US
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
PURDUE RESEARCH FOUNDATION 1291 Cumberland Avenue West Lafayette, IN 47906 United States of America		
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Leitao
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

003597367

Express Mail No. EL230257915US Rec'd 11/8/01

PATENT COOPERATION TREATY 09/857307

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: STEVEN R. LAMMERT
BARNES & THORNBURG
11 SOUTH MERIDIAN STREET
INDIANAPOLIS IN 46204

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	12 JAN 2001
Applicant's or agent's file reference: 3220-65477	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US99/28300	01 DECEMBER 1999	01 DECEMBER 1998

Applicant
PURDUE RESEARCH FOUNDATION

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer DAVID J ISABELLA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-3060

Form PCT/IPEA/416 (July 1992)*

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 3220-65477	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/28300	International filing date *(day/month/year)* 01 DECEMBER 1999	Priority date *(day/month/year)* 01 DECEMBER 1998
International Patent Classification (IPC) or national classification and IPC IPC(7):A61B 19/00 and US Cl.: 128/898		
Applicant PURDUE RESEARCH FOUNDATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08 JUNE 2000	12 DECEMBER 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID J ISABELLA Telephone No. (703) 308-3060

Form PCT/IPEA/409 (cover sheet) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/28300

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-16, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the claims:
pages 17-18, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the drawings:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Int[redacted]onal application No.
PCT/US99/28300

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-11	YES
	Claims	NONE	NO
Inventive Step (IS)	Claims	1-11	YES
	Claims	NONE	NO
Industrial Applicability (IA)	Claims	1-11	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-11 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest a surgical method for repair of the vocal cord tissues by replacing the damaged tissue with a graft construct derived from the vertebrate submucosa or basement membrane.

---------- NEW CITATIONS ----------

US 5,549,673 A (BEALE) 27 AUGUST 1996, ENTIRE PUBLICATION

US 5,762,966 A (KNAPP, JR. ET AL) 9 JUNE 1998, ENTIRE PUBLICATION

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61L 27/38

A1

(11) International Publication Number: WO 00/32254

(43) International Publication Date: 8 June 2000 (08.06.00)

(21) International Application Number: PCT/US99/28300

(22) International Filing Date: 1 December 1999 (01.12.99)

(30) Priority Data:

60/110,401	1 December 1998 (01.12.98)	US
60/110,465	1 December 1998 (01.12.98)	US

(71) Applicant *(for all designated States except US)*: PURDUE RESEARCH FOUNDATION [US/US]; 1021 Hovde Hall, Room 307, West Lafayette, IN 47907–1021 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BADYLAK, Stephen, F. [US/US]; 1150 Kingswood Road South, West Lafayette, IN 47906 (US). SPIEVACK, Alan, R. [US/US]; 6 Old Dee Road, Cambridge, MA 02138 (US).

(74) Agent: LAMMERT, Steven, R.; Barnes & Thornburg, 11 South Meridian Street, Indianapolis, IN 46204 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR VOCAL CORD RECONSTRUCTION

(57) Abstract

A method for surgical repair of damaged or diseased head and neck tissues is described. In one aspect of the invention tissue graft constructs comprising vertebrate submucosa or vertebrate basement membrane materials are used to repair and promote growth of endogenous vocal cord tissue.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
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EE	Estonia	**LR**	Liberia	**SG**	Singapore		

METHOD FOR VOCAL CORD RECONSTRUCTION

Field of the Invention

The present invention relates to a tissue graft and method for repairing damaged or diseased head and neck soft tissues. More particularly, this invention is directed to a method for promoting growth of endogenous vocal cord tissue to repair damaged or diseased vocal cords.

Background and Summary of the Invention

There is a significant need for suitable scaffold materials in reconstructive surgery of the head and neck region. Congenital and acquired deformations of structures such as larynx, soft and hard palate, nasal, auricular, and facial bones are common, and biomaterials available for surgical repair of these objects are limited. Contracture, infection, and poor integration into the surrounding tissues are frequent problems with such materials. Clearly a tissue graft material is desired which is non-immunogenic, is not subject to gross shrinkage after implantation, and promotes the growth of endogenous vocal cord, larynx, soft and hard palate, nasal, and auricular tissues.

The naturally-occurring extracellular matrix (ECM) of the small intestinal submucosa, as well as other vertebrate sources of submucosa, has been shown to serve as a resorbable scaffold for numerous body systems. Surprisingly, it too has been found that basement membranes (stroma) prepared from liver tissue of warm-blooded vertebrates (by removing cellular components of the liver tissue) exhibit mechanical and biotropic properties suitable for use as a tissue graft material. The present invention is directed to the use of vertebrate submucosa matrices and basement membranes as tissue grafts for replacing damaged or diseased portions of head and neck soft tissue and promoting the remodeling and regeneration of the tissue graft with endogenous tissues. The submucosa matrices used in accordance with the present invention comprises highly conserved collagens, glycoproteins, proteoglycans, and glycosaminoglycans in their natural configuration and natural concentration. Vertebrate submucosa is a relatively acellular collagen-based matrix that can be isolated from animal tissues, including particularly intestinal tissue harvested from animals raised for meat production. The isolated

submucosa can be used to prepare a resorbable tissue graft construct for inducing the repair of endogenous tissues.

The basement membrane graft compositions of the present invention comprise the basement membrane of organ tissue of a warm-blooded vertebrate, for example, liver tissue, substantially free, preferably devoid, of all cells (*e.g.*, hepatocytes and bile ductal cells) of said warm-blooded vertebrate. The liver basement membrane can be implanted, or fluidized and injected, into a vertebrate host to contact damaged or defective vocal cord, larynx, soft and hard palate nasal and auricular tissues and induce the repair or replacement of said tissues *in vivo*.

It is known that compositions comprising the tunica submucosa and the basilar portions of the tunica mucosa of the intestine of warm-blooded vertebrates can be used as tissue graft materials in sheet form. See U.S. Patent No. 4,902,508. The compositions described and claimed in that patent are characterized by excellent mechanical properties, including high compliance, a high burst pressure point, and an effective porosity index which allows such compositions to be used beneficially for vascular graft constructs. The graft materials disclosed in that patent are also useful in tendon, ligament and other connective tissue replacement applications. Furthermore, intestinal submucosa has been used as a scaffold for regenerating other tissues including urinary bladder and dura mater. When used in such applications the preferred graft constructs appear to serve as a matrix for the regrowth of the tissues replaced by the graft constructs. Vertebrate submucosa is a plentiful by-product of commercial meat production operations and is thus a low cost tissue graft material, especially when the submucosa is used in its native sheet configuration. Intestinal submucosa has undergone extensive immunologic testing in over 600 cross-species implants and has never been shown to elucidate a rejection reaction.

Furthermore, it is known that intestinal submucosa can be fluidized by comminution and/or protease digestion, without loss of its apparent biotropic properties, for use in less invasive methods of administration (e.g., injection or topical) to host tissues in need of repair. See U.S. Patent No. 5,275,826 the disclosure of which is expressly incorporated herein. Fluidized comminuted intestinal tissue comprising tunica submucosa has previously been successfully used to repair and functionally augment damaged tissues including, for example, urinary bladder sphincter. Common events to tissue remodeling

include widespread and rapid neovascularization, proliferation of granulation mesenchymal cells, biodegradation of implanted submucosa, and lack of immune rejection.

The present invention is directed to the use of vertebrate-derived submucosa or basement membrane matrices as a graft for the regeneration and repair of head and neck soft tissues including the larynx, vocal cords, soft and hard palate, attached gingiva, nasal and auricular tissues. Such vertebrate extracellular matrices are inexpensive, nonimmunogenic materials that induce host tissue proliferation, remodeling and regeneration upon implantation. In accordance with one embodiment of the present invention tissue graft constructs comprising submucosa or basement membrane of a warm-blooded vertebrate have been found to promote the growth of endogenous vocal cord tissues including the oral mucosal epithelium, connective tissue and skeletal muscle. The graft constructs of the present invention can be used to repair or reconstruct structures damaged by cancer or resulting from congenital defects. The method comprises replacing the damaged or diseased tissues with the construct which acts as a scaffold for endogenous cell growth and replacement of the graft construct. The scaffold is typically entirely replaced by endogenous tissues in about three to six weeks.

Detailed Description of the Invention

There is provided in accordance with the present invention a method and composition for repairing damaged or diseased head and neck soft tissues including the vocal cord, larynx, soft and hard palate, attached gingiva, nasal and auricular tissues. The extracellular matrix graft compositions function as a biotropic/biodegradable scaffold that induces endogenous tissues to invade and replace the graft material with endogenous tissue. After implantation, the constructs are eventually remodeled by the host with tissues having a stratification of cell layers similar to that found in normal endogenous tissues.

One tissue graft construct used in accordance with the present invention is derived from vertebrate submucosa and comprises naturally associated extracellular matrix proteins, glycoproteins and other factors. Suitable submucosa comprises the tunica submucosa delaminated from the tunica muscularis and at least the luminal portion of the tunica mucosa. Preferably, the submucosa comprises intestinal submucosa of a

warm-blooded vertebrate, and one particularly preferred source of the submucosa is the small intestine of warm-blooded vertebrates. In accordance with one embodiment of the present invention the submucosa is intestinal submucosa comprising the tunica submucosa and basilar portions of the tunica mucosa including the lamina muscularis mucosa and the stratum compactum which layers are known to vary in thickness and in definition dependent on the source vertebrate species. Submucosa can also be prepared from other organs of vertebrate species, for example, from the urogenital system, including the urinary bladder (see U.S. Patent Nos. 5,554,389), and other portions of the digestive tract including the stomach (see published PCT application no. WO98/25636). The disclosures of U.S. Patent Nos. 5,554,389 and published PCT application no. WO98/25636 are expressly incorporated herein.

The preparation of vertebrate submucosa for use in accordance with this invention is described in U.S. Patent Nos. 4,902,508 and 5,554,389. To summarize, submucosa is prepared from vertebrate intestine (or other organ source), preferably harvested from porcine, ovine or bovine species, but not excluding other species, by subjecting the intestinal tissue to abrasion using a longitudinal wiping motion to remove the outer layers, comprising smooth muscle tissues, and the innermost layer, *i.e.*, at least the luminal portion of the tunica mucosa. The submucosa is rinsed with saline and optionally sterilized; it can be stored in a hydrated or dehydrated state. Lyophilized or air dried vertebrate submucosa can be rehydrated and used in accordance with this invention without significant loss of its cell proliferative activity. Native submucosa as a starting material is a relatively acellular collagenous matrix and the process of preparing intestinal submucosa for use as the collagenous matrix component of the present invention produces a collagenous matrix devoid of intact cells. Accordingly the submucosa collagenous matrix prepared in accordance with the present invention is acellular.

It is known that compositions comprising the tunica submucosa of the intestine of warm-blooded vertebrates can be used advantageously as tissue graft materials. See U.S. Patent Nos. 4,902,508 and 5,281,422, the disclosures of which are expressly incorporated herein by reference. The tissue graft compositions described in those patents are used beneficially for vascular graft and connective tissue graft constructs. When used in such applications the graft constructs appear not only to serve as a matrix for the regrowth of the tissues replaced by the graft constructs, but also

promote or induce such regrowth of endogenous tissue. Common events to this remodeling process include widespread and rapid neovascularization, proliferation of granulation mesenchymal cells, biodegradation/resorption of implanted submucosa, and lack of immune rejection.

It is also known that intestinal submucosa can be fluidized by comminuting and/or enzymatic digestion, without loss of its apparent biotropic properties, for use in less invasive methods of administration (e.g., by injection or topical application) to host tissues in need of repair. See U.S. Patent No. 5,275,826, the disclosure of which is expressly incorporated herein by reference.

In another embodiment of the invention the tissue graft composition comprises liver basement membrane prepared by separating same from the natively associated cellular components of liver tissue of a warm-blooded vertebrate. The preparative techniques described below provide an extracellular matrix composition consisting essentially of liver basement membrane substantially free of any cellular components. These compositions are referred to herein generically as liver basement membrane(s) (LBM). Other organ tissue sources of basement membrane for use in accordance with this invention include spleen, lymph nodes, salivary glands, prostate, pancreas and other secreting glands.

Basement membrane for preparation of the graft compositions used in accordance with this invention is typically prepared from liver tissue harvested from animals raised for meat production, including, for example, pigs, cattle and sheep or other warm-blooded vertebrates. Thus, there is an inexpensive commercial source of liver tissue for use in preparation of the basement membrane derived tissue graft compositions for use in accordance with the present invention. In one embodiment, a composition comprising liver basement membranes is prepared from liver tissue of a warm-blooded vertebrate. This composition is useful in accordance with this invention as a non-immunogenic tissue graft capable of inducing endogenous tissue growth when implanted in warm-blooded vertebrates. In one embodiment, the composition comprises an extracellular matrix consisting essentially of liver basement membrane devoid of endogenous cells associated with the source vertebrate liver tissue used to prepared the composition.

The preparation of liver basement membrane from liver tissue of a warm-blooded vertebrate is carried out by removing the cellular components from liver tissue. Ideally the process is carried out to separate the cells from the basement membranes without damaging, or at least minimizing disruption or damage to, the basement membrane tissue. Removal of the cellular components from the liver extracellular matrix allows the preparation of a graft composition that is non-immunogenic, and thus does not induce a host immune response when the graft composition is implanted into a host. In general, the method for preparing a tissue graft composition from warm-blooded vertebrate liver tissue comprises the steps of treating the liver tissue with a cell dissociation solution for a period of time sufficient to release the cellular components of the liver tissue from the extracellular components without substantial disruption of the extracellular components, and separating the cellular components from said extracellular components. Typically the cell dissociation solution comprises a chaotropic agent or an enzyme or both.

The first step in preparing liver basement membrane for use in accordance with one embodiment of the present invention comprises slicing a segment of liver tissue into pieces (*e.g.*, strips or sheets) to increase the surface area-to-volume ratio of the liver tissue. In one embodiment the liver tissue is sliced into a series of sheets each having a thickness of about 0.05 to about 1.5 mm, more particularly, about 50 to about 500 microns, and more preferably about 250 to about 300 microns. Freshly harvested liver tissue can be sliced using a standard meat slicer, or the tissue can be frozen and sliced with a cryomicrotone. The thin pieces of liver tissue are then treated with a solution that releases component liver cells from the associated extracellular basement membrane matrix.

The liver tissue can be also treated with a solution comprising an enzyme, for example, a protease, such as trypsin or pepsin. Because of the collagenous structure of the liver basement membrane and the desire to minimize degradation of the membrane structure during cell dissociation, collagen specific enzyme activity should be minimized in the enzyme solutions used in the cell-dissociation step. In addition, the liver tissue is typically also treated with a calcium chelating agent or chaotropic agent such as a mild detergent such as Triton 100. Thus, in one embodiment of this invention liver tissue is treated by suspending slices or strips of the tissue in a cell-dissociation solution containing

enzyme(s) and chaotropic agent(s). However, the cell dissociation step can also be conducted using a calcium chelating agent or chaotropic agent in the absence of an enzymatic treatment of the tissue.

In preparative method the cell-dissociation step is carried out by suspending liver tissue slices in an agitated solution containing about 0.05 to about 2%, more typically about 0.1 to about 1% by weight protease, optionally containing a chaotropic agent or a calcium chelating agent in an amount effective to optimize release and separation of cells from the basement membrane without substantial degradation of the membrane matrix.

After contacting the liver tissue with the cell-dissociation solution for a time sufficient to release all cells from the matrix, the resulting liver basement membrane is rinsed one or more times with saline and optionally stored in a frozen hydrated state or a partially dehydrated state until used as described below. The cell-dissociation step may require several treatments with the cell-dissociation solution to release substantially all cells from the basement membrane. In one embodiment liver tissue is treated with a protease solution to remove the component cells, and the resulting extracellular matrix material (basement membrane) is further treated to remove or inhibit any residual enzyme activity. For example, the resulting basement membrane can be heated or treated with one or more protease inhibitors.

Liver basement membrane for use in carrying out this invention can be fluidized (converted to an injectable or powder form) in a manner similar to the preparation of fluidized intestinal submucosa, as described in U.S. Patent No. 5,275,826 the disclosure of which is expressly incorporated herein by reference.

In accordance with one embodiment of the present invention a multi-layered submucosa or basement membrane construct is formed from multiple sheets/strips of submucosa and/or basement membrane. The method of forming the multi-layered construct comprises the steps of overlapping multiple sheets of submucosa and/or basement membrane and adhering the layers to each other. The individual layers can be fix to one another using standard techniques know to those skilled in the art and including the use of sutures, staples and biocompatible adhesives such as collagen binder pastes. In one embodiment the layers are fused together by compressing the overlapped regions under dehydrating conditions, optionally with the addition of heat.

The individual layers forming the multi-layered construct can be prepared from sheets of submucosa and/or basement membrane, wherein each sheet is cut to the same dimensions. Alternatively each sheet of the multilayered construct may be cut to have different dimensions, and in one embodiment the sheets comprising the multi-layered construct may have the same width and length but may differ in thickness. Typically the sheets of basement membrane will be cut to have a thickness of about 0.05 mm to about 1.5 mm, and more preferably about 0.2 to about 0.5 mm.

In one embodiment of the present invention, a first strip of submucosa or basement membrane can be partially overlapped with a second strip of submucosa or basement membrane and the two strips adhered to one another to form a large area, graft construct as described in US Patent No. 5,711,969, the disclosure of which is expressly incorporated herein. The process of forming large area graft sheets involves cutting strips of submucosa and overlapping at least a portion of each strip with a portion of an adjacent strip. The overlapped regions are then adhered to one another using techniques known to those skilled in the art. Alternatively, consecutive layers of extracellular matrix material (submucosa and/or basement membrane) can be layered on top of one another so that each layer is entirely covered by the second layer, thus generating a multi-layered construct uniform in thickness throughout the graft construct. In one embodiment the multi-layered constructs are perforated to allow fluids to readily pass through the graft construct and prevent pockets of fluids from accumulating between the layers. The formation of perforated multilayered constructs is described in US Patent No, 5,755,791, the disclosure of which is expressly incorporated herein.

In one embodiment, the overlapped portions are compressed under dehydrating conditions to fuse the overlapped portions to one another and form a large sheet. In one preferred embodiment, a multi-layered graft construct is prepared without the use of adhesives or chemical pretreatments by compressing at least the overlapped portions of extracellular matrix under conditions that allow dehydration of the material concurrent with the compression of the tissue. To promote dehydration of the compressed material, at least one of the two surfaces compressing the tissue is water permeable. Dehydration can optionally be further enhanced by applying blotting material, heating the material or blowing air across the exterior of the two compressing surfaces.

In one embodiment the method of forming the multi-layered construct comprises layering the strips onto a permeable surface and using a second optionally permeable surface to compress the overlapped portions between the two surfaces. In one embodiment, strips are organized on a mesh in one direction with at least a portion of one strip overlapping with at least a portion of another strip. Once the mesh is covered with one layer of extracellular matrix material a second layer is applied on top of the first layer but at a different angle relative to the first layer. Additional layers can be added to obtain a graft construct having a desired strength or thickness.

After all the strips are placed on the mesh, another mesh is placed on top of the layers and the "mesh-tissue layers-mesh" sandwich is compressed with a load and dried. This process produces a dried large area construct that can be pealed off the mesh.

In one embodiment the graft construct is formed from two or more strips of extracellular matrix material pressed together and dried through the use of vacuum bagging. In that method submucosa or basement membrane is laid out between two perforated, preferably stainless steel, plates. The plates are shaped to define the desired shape, e.g. two concentric cylinders are used to form a multilayered tubular construct. The material is optionally placed on a surface and covered with blotting material to soak up water, and a breather blanket to allow air flow. The resulting "sandwich" of pressure plates and matrix material is then sealed into a nylon bag that has a vacuum port. A vacuum is applied to pull air out of the vacuum bag and the resulting drop in atmospheric pressure compresses the plates against the matrix material and simultaneously, at least partially, dehydrates the material. After 4 to 24 hours of applying a vacuum, the produced sheet is still moist and very flexible. No seams from the layering are visible and the strength of a prototype 8-thickness sheet as determined by ball burst test is approximately 80 pounds. This general procedure can also be used to shape single tissue strips for use in this invention, if "shaping" of such single layer tissue constructs is determined to be necessary or appropriate for particular surgical application.

In one embodiment, during formation of the large area sheets of tissue, pressure is applied to the overlapped portions under dehydrating conditions by compressing the overlapped tissue segments between two surfaces. The two surfaces can be formed from a variety of materials and in any shape, depending on the desired form and specification of the targeted graft construct. Typically the two surfaces are formed as

flat plates but they can also include other shapes such as screens, opposed cylinders or rollers and complementary nonplanar surfaces. Each of these surfaces can optionally be heated or perforated. In preferred embodiments at least one of the two surfaces is water permeable. The term water permeable surface as used herein includes surfaces that are water absorbent, microporous or macroporous. Macroporous materials include perforated plates or meshes made of plastic, metal, ceramics or wood.

Alternatively, large area sheets extracellular matrix graft material can be formed from smaller segments of graft material through the use of sutures and/or the use of binder pastes as described in U.S. Patent No. 3,562,820, the disclosure of which is expressly incorporated herein by reference. The mechanical properties of the large area grafts can be altered by adjusting the number of layers in the sheet, varying the angle of adjacent layers to each other, and varying the load applied to press the component tissue strips into a large area sheet.

The vertebrate submucosa used in the present invention can be conditioned to alter the viscoelastic properties of the submucosa by stretching the material in a longitudinal or lateral direction as described in U.S. Patent No. 5,275,826, the disclosure of which is expressly incorporated herein by reference. In accordance with one embodiment submucosa delaminated from the tunica muscularis and luminal portion of the tunica mucosa is conditioned to have a strain of no more than 20%. The submucosa is conditioned by stretching, chemically treating, enzymatically treating or exposing the tissue to other environmental factors. In one embodiment the strips of intestinal submucosa tissue are conditioned by stretching in a longitudinal or lateral direction so that the strips of intestinal submucosa tissue have a strain of no more than 20%.

In one embodiment the submucosa is conditioned by stretching the graft material longitudinally to a length longer than the length of the submucosa from which the graft construct was formed. One method of conditioning the tissue by stretching involves application of a given load to the submucosa for three to five cycles. Each cycle consists of applying a load to the graft material for five seconds, followed by a ten second relaxation phase. Three to five cycles produces a stretch-conditioned graft material with reduced strain. The graft material does not immediately return to its original size; it remains in a "stretched" dimension. Optionally, the graft material can be preconditioned by stretching in the lateral dimension.

In one embodiment the submucosa is stretched using 50% of the predicted ultimate load. The "ultimate load" is the maximum load that can be applied to the submucosa without resulting in failure of the tissue (i.e. the break point of the tissue). Ultimate load can be predicted for a given strip of submucosa based on the source and thickness of the material. Accordingly, one method of conditioning the tissue by stretching involves application of 50% of the predicted ultimate load to the submucosa for three to ten cycles. Each cycle consists of applying a load to the graft material for five seconds, followed by a ten second relaxation phase. The resulting conditioned submucosa has a strain of less than 30%, more typically a strain from about 20% to about 28%. In one preferred embodiment conditioned the submucosa has a strain of no more than 20%. The term strain as used herein refers to the maximum amount of tissue elongation before failure of the tissue, when the tissue is stretched under an applied load. It is expressed as a percentage of the length of the tissue before loading. The conditioned submucosal strips can be used to form a graft construct of the present invention or alternatively the graft construct can be conditioned after its formation. For the multi-layered constructs the submucosa can be stretched prior to the formation of the graft construct, during the formation of the construct, or the submucosa can be stretched after formation of the multi-layered construct.

The graft compositions of the present invention can be sterilized using conventional sterilization techniques including glutaraldehyde tanning, formaldehyde tanning at acidic pH, ethylene oxide treatment, propylene oxide treatment, gas plasma sterilization, gamma radiation, electron beam and peracetic acid sterilization. Sterilization techniques which do not adversely affect the mechanical strength, structure, and biotropic properties of the graft constructs are preferred. For instance, strong gamma radiation may cause loss of strength of the sheets. Preferred sterilization techniques include exposing the graft to peracetic acid, 1-4 Mrads gamma irradiation (more preferably 1-2.5 Mrads of gamma irradiation) or gas plasma sterilization; peracetic acid sterilization is the most preferred sterilization method. Typically, the graft construct is subjected to two or more sterilization processes. After sterilization, for example by chemical treatment, the tissue graft construct may be wrapped in a plastic or foil wrap and sterilized again using electron beam or gamma irradiation sterilization techniques.

There is provided in accordance with the present invention a method and composition for repairing damaged or diseased head and neck soft tissues including the vocal cord, larynx, soft and hard palate, attached gingiva, nasal and auricular tissues. The above described graft compositions function as a biotropic/biodegradable scaffold that induces endogenous tissues to invade and replace the graft material with endogenous tissue. Advantageously the graft constructs induce the proliferation of endogenous cells to form native tissues of the native structure, including an epithelial cell layer, connective tissue and functional muscle.

In accordance with one embodiment of the present invention, vertebrate submucosa or basement membrane material is used as a tissue graft for reconstructing damaged or diseased larynx and vocal cord tissues. In one embodiment a damaged or diseased section of the vocal cord, or even the entire vocal cord, is removed and replaced with a tissue graft construct as described above. The tissue graft induces the growth of endogenous vocal cord tissues, including oral mucosal epithelial cells, and functional skeletal muscles, and thus promotes the repair of the damaged or diseased tissues. The method of repair comprises the steps of surgically removing the damaged or diseased portion and replacing the removed portion with a tissue graft construct comprising submucosa or basement membrane of a warm-blooded vertebrate. Controls indicate that in the absence of the present graft material, severed vocal cords form scar tissue at the wound site and fail to regenerate the severed vocal cord.

In one embodiment submucosa used for the repair of head and neck soft tissues is isolated from intestinal tissue and comprises the tunica submucosa delaminated from both the tunica muscularis and at least the luminal portion of the tunica mucosa. Alternatively, the submucosa can be prepared from urinary bladder or stomach tissues. In accordance with one embodiment the tissue graft construct comprises multiple layers of vertebrate submucosa comprising 2-12 layers of submucosa, more preferably 4-6 layers. The multi-layered construct in one embodiment comprises partially overlapped strips of submucosa and more preferably the tissue graft construct is formed as a multilayered homolaminate (i.e. having the same number of layers throughout the graft) construct. Basement membrane material can be used similarly alone or in combination with submucosa tissue.

In accordance with one embodiment of the present invention, there is provided a method for reconstructing diseased or damaged vocal cord tissues. The method comprises the steps of surgically removing the damaged or diseased vocal cord tissues and replacing the removed tissues with a tissue graft construct comprising an extracellular matrix of a warm-blooded vertebrate. In one embodiment the entire vocal cord is removed and replaced with submucosa tissue or basement membrane tissue or some combination thereof. The graft construct serves as a scaffold for inducing the proliferation and repair of the vertebrate vocal cords. The graft is remodelled within about three to six weeks forming functional skeletal muscle, an oral mucosal epithelial layer and supporting connective tissue. The tissue graft constructs can be implanted into a vertebrate host species to repair a damaged, diseased or otherwise functionally compromised vocal cord. The xenogeneic materials do not elicit any adverse immune response or adverse inflammatory reaction. The scaffolds appear to be rapidly resorbed and replaced by varying amounts of host connective tissues without shrinkage of the graft area or formation of "scar" tissue.

In one embodiment the defective portion of the larynx or vocal cord is surgically removed and replaced with a tissue graft construct comprising submucosa of a warm-blooded vertebrate. Where the submucosa is of intestinal origin it is preferred that the luminal side of the intestinal submucosa is directed toward the larynx lumen. Large portions of the larynx can be removed and replaced with the tissue grafts of the present invention. After implantation, the constructs are eventually remodelled by the host with functional larynx tissues having a stratification of cell layers similar to that found in the normal larynx wall.

It is anticipated that vertebrate submucosa and/or basement membrane is capable of inducing host tissue proliferation, remodeling and regeneration of appropriate tissue structures upon implantation in a number of microenvironments *in vivo* (e.g. soft tissues of the head and neck, including the larynx, vocal cords, soft and hard palate, attached gingiva, nasal and auricular tissues). In one embodiment of the present invention the tissue replacement capabilities of graft compositions comprising vertebrate submucosa or basement membrane of warm-blooded vertebrates are further enhanced or expanded by seeding the tissue with various cell types, prior to implantation. For example, a submucosa construct may be seeded with mesenchymal cells (stem cells) initially for

expansion of the cell population and thereafter for implantation into a host. In accordance with one embodiment the constructs are seeded with epithelial cells before implantation of the graft construct. In accordance with another embodiment epithelial cells are first cultured on one side of the graft construct and then muscle cells are cultured on the opposite side of the graft construct before the graft is implanted.

Example 1

Preparation of intestinal submucosa

Small intestine submucosa was prepared in accordance with the procedures described in U.S. Patent No. 4,902,508. Briefly, sections of porcine jejunum were harvested within ten minutes of euthanasia and immediately placed in 0.9% saline solution. These sections were cut into 10 to 20 cm lengths and the mesenteric tissues were removed from the segment of the small intestine. The small intestine was exerted (inside out) and the tunica mucosa mechanically removed. The small intestinal segment was exerted again (i.e. the stratum compactum on the luminal side, as in the original orientation) and the serosa and tunica muscularis were removed from the outer surface. The tissue was rinsed in saline and placed in a 10% neomycin sulfate solution until used as a graft material. Storage time for the graft material ranged from 2 weeks to 3 months. It should be noted that preparation of submucosa is a mechanical process similar to that of sausage casing and involves no enzymatic reaction steps.

Example 2

Surgical Repair of vocal cords

Materials and Methods: Seven healthy adult female mongrel dogs were subjected to bilateral resection of the vocal folds. One side was repaired with a single thickness sheet of either intestinal submucosa or urinary submucosa both of which are resorbable naturally-occurring scaffolds. The contralateral side in each dog was left unfilled as a control. The dogs were evaluated at time points ranging from three weeks to several months.

Results: At three weeks, there was significant remodeling along the framework of the resorbable scaffolds. Deposition of new extracellular matrix, an abundant vascular

component, and a dense infiltration of mononuclear cells existed within the space occupied by the original graft construct. At three weeks, none of the graft constructs could be identified with either routine H&E staining or Masson's Trichrome staining. There was a subtotal epithelialization of the surface of each of these grafts. The contralateral (control) side showed scar tissue formation partially filling the defect. Macroscopic and microscopic results of the longer surviving dogs are in preparation.

Example 3

Preparation of Liver Basement Membrane

2 mM EDTA Chaotropic Solution Used In The Experiment

140mM	NaCl
5mM	KCl
0.8mM	$MgSO_4$
0.4mM	KH_2HPO_4
2mM	EDTA
25mM	$NaHCO_3$

Procedure:

Preparation of liver slices:

Liver frozen in -70°C was sliced with a cryomicrotone to a thickness of about 50μM. The slices of liver tissue were then subjected to enzymatic treatment (trypsin) with a chaotropic solution (samples 1 and 2), with enzyme alone (samples 3 and 4), or with a chaotropic solution alone (sample 5), as indicated below.

Sample #	Treatment
1)	0.05% Trypsin in 2mM EDTA solution
2)	0.1% Trypsin in 2mM EDTA solution
3)	0.05% Trypsin in 2mM PBS
4)	0.1% Trypsin in 2mM PBS
5)	2mM EDTA solution

Liver slices were placed in five 50ml tubes, each of which contained 25mL of a different buffered enzyme treatment solution. The liver tissue was incubated at 37°C

in water bath with gentle shaking for 1 hour. The liver slices were washed twice with PBS with agitation/shaking for 1 hour at room temperature. The above enzymatic treatment steps were repeated three times.

The wash buffers were collected and spin them down in 2000rpm for 10 min. The pellet was suspended and an equal amount of trypan blue was added to identify any remaining cells. The material was checked for presence of cells under microscope.

Example 4

Mechanical Properties of Isolated Liver Basement Membrane

Porosity of a graft material is typically measured in terms of ml of water passed per cm^2min^{-1} at a pressure of 120 mm Hg. The average "porosity index" established for two separate specimens of LBM was 1162. The suture retention strength of LBM is approximately 68 grams. The material appears to be anisotropic, with the suture strength being approximately the same in all directions.

Example 5

Surgical Repair of vocal cords

Materials and Methods: Seven healthy adult female mongrel dogs were subjected to bilateral resection of the vocal folds. One side was repaired with a single thickness sheet of LBM which is a resorbable naturally-occurring scaffold. The contralateral side in each dog was left unfilled as a control. The dogs were evaluated at time points ranging from three weeks to several months.

Results: At three weeks, there was significant remodeling along the framework of the resorbable scaffolds. Deposition of new extracellular matrix, an abundant vascular component, and a dense infiltration of mononuclear cells existed within the space occupied by the original graft construct. At three weeks, none of the graft constructs could be identified with either routine H&E staining or Masson's Trichrome staining. There was a subtotal epithelialization of the surface of each of these grafts. The contralateral (control) side showed scar tissue formation partially filling the defect. Macroscopic and microscopic results of the longer surviving dogs are in preparation.

CLAIMS:

1. A method for the repair or replacement of vocal cord tissues comprising the steps of:

removing the a damaged or diseased portion of a vocal cord, and

replacing the removed portion of a vocal cord with a graft construct comprising vertebrate submucosa or basement membrane.

2. The method of claim 1 wherein the graft comprises submucosa and the submucosa is selected from the group consisting of intestinal submucosa, urinary bladder submucosa, and stomach submucosa.

3. The method of claim 2 wherein the submucosa is intestinal submucosa and comprises the tunica submucosa delaminated from the tunica muscularis and the luminal portion of the tunica mucosa.

4. The method of claim 1 wherein the graft construct comprises vertebrate basement membrane.

5. The method of claim 1 wherein the graft construct comprises 2-12 layers of submucosa.

6. The method of claim 1 wherein the graft construct comprises 4-6 layers of submucosa.

7. The method of claim 5 wherein the graft construct is formed as a multilayered homolaminate.

8. The method of claim 1 wherein the graft construct comprises a single thickness sheet of submucosa.

9. A method for the repair or replacement of damaged or diseased head and neck soft tissues comprising the steps of

removing the a damage or diseased portion of the diseased or damaged tissue, and

replacing the removed portion of tissue with a graft construct comprising vertebrate submucosa or basement membrane.

10. The method of claim 8 wherein the head and neck soft tissues are selected from the group consisting of vocal cord, larynx, palette, attached gingiva, nasal, and auricular tissues.

11. The use of vertebrate submucosa or vertebrate basement membrane to manufacture a non-immunogenic tissue graft composition for repairing vocal cords and other soft tissues of the head and neck.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PC 99/28300

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 10775 A (BADYLAK STEPHEN F ;COBB MARK A (US); ISOM GARY (US); SHARMA ARCHAN) 19 March 1998 (1998-03-19)	9,11
A	example 2 claims	1-3,5-8, 11
Y	ISSHIKI N ET AL: "Surgical treatment of laryngeal web with mucosa graft" ANNALS OF OTOLOGY, RHINOLOGY AND LARYNGOLOGY, vol. 100, 1991, pages 95-100, XP000901865 page 95, column 2, line 10 - line 16 page 99, column 2, last paragraph figure 2	1-11

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 April 2000	26/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Thornton, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter[redacted]onal Application No
PCT[redacted]99/28300

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 25637 A (BADYLAK STEPHEN F ;PURDUE RESEARCH FOUNDATION (US)) 18 June 1998 (1998-06-18) page 3, line 3 -page 5, line 2 page 6, line 4 - line 9 page 12, line 3 -page 13, line 6 claims	1-11
A	US 5 573 784 A (BADYLAK STEPHEN F ET AL) 12 November 1996 (1996-11-12) column 1, line 16 - line 55 claim 1	1-3,5-11
A	WO 98 40027 A (GERIGENE MEDICAL CORP ;KLEINSEK DON A (US)) 17 September 1998 (1998-09-17) page 28, line 15 -page 29, line 25 claims	1,9-11
A	WO 96 40175 A (ADVANCED TISSUE SCIENCES INC) 19 December 1996 (1996-12-19) page 50, line 19 -page 53, line 16 claims 1-6,10	1,5-11
A	PANKRATOV M ET AL: "Endoscopic diode-laser applications in airway surgery" PROC SPIE INT SOC OPT ENG. PROCEEDINGS OF SPIE - THE INTERNATIONAL SOCIETY FOR OPTICAL ENGINEERING. PROCEEDINGS OF LASER SURGERY: ADVANCED CHARACTERIZATION, THERAPEUTICS, AND SYSTEMS IV, vol. 2128, 1994, pages 33-40, XP000901390 ISSN 0277-786X ISBN 0-8194-1421-2 page 33, last paragraph -page 34, line 10 page 37, line 24 -page 38, line 16 page 38, last paragraph	1,9,10

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PC[redacted] 99/ 28300

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-10 are directed to a method of treatment of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box 1.1

Although claims 1-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box 1.1

Rule 39.1(iv) PCT - Method for treatment of the humanr/animal body by surgery

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/99/28300

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